

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1. (Withdrawn) A polynucleotide selected from the group consisting of:
  - (a) a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO: 1;
  - (b) a polynucleotide encoding a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2;
  - (c) a polynucleotide hybridizing to a DNA comprising the nucleotide sequence set forth in SEQ ID NO: 1 under a stringent condition, wherein said polynucleotide encodes a polypeptide having the activity of a D-aminoacylase having the physicochemical properties of (i) and (ii) below; and
  - (d) a polynucleotide encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO: 2 in which one or more amino acid are substituted, deleted, inserted, and/or added, wherein said polynucleotide encodes a polypeptide having the activity of a D-aminoacylase having the physicochemical properties of (i) and (ii) below
    - (i) action: the enzyme acts on N-acetyl-D-amino acids to produce the corresponding D-amino acids and
    - (ii) substrate specificity: the enzyme acts on N-acetyl-D-tryptophan, N-acetyl-D-phenylalanine, N-acetyl-D-valine, N-acetyl-D-leucine, and N-acetyl-D-methionine, but not on N-acetyl-L-tryptophan, N-acetyl-L-phenylalanine, N-acetyl-L-valine, N-acetyl-L-leucine, or N-acetyl-L-methionine.
2. (Withdrawn) A polypeptide encoded by the polynucleotide of claim 1.
3. (Withdrawn) A vector comprising the polynucleotide of claim 1.
4. (Withdrawn) A transformed host cell comprising the polynucleotide of claim 1.

5. (Withdrawn) The transformed host cell of claim 4, wherein said cell is derived from *E. coli*.

6. (Withdrawn) A method of producing a polypeptide, said method comprising culturing the transformed host cell of claim 4 in a culture, expressing the polypeptide in the cell, and recovering the polypeptide from the culture.

7. (Withdrawn) The method of claim 6, wherein said cell is derived from *E. coli*.

8. (Withdrawn) A polynucleotide hybridizing to the polynucleotide set forth in SEQ ID NO: 1 or the complement thereof, wherein said polynucleotide comprises at least 15 nucleotides.

9. (Withdrawn) A method for synthesizing a polynucleotide, said method comprising chemically synthesizing the polynucleotide of claim 8.

10. (Withdrawn) A method for detecting a polynucleotide, said method comprising hybridizing the polynucleotide of claim 8 to a test polynucleotide, and determining whether hybridization has occurred.

11 (Currently Amended) A method for producing D-amino acids, wherein said method comprising comprises the steps of:

(1) contacting a polypeptide selected from the group consisting of:

(a) a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2;

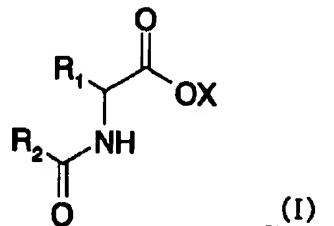
(b) a polypeptide consisting of the amino acid sequence set forth in SEQ ID NO:2;

(c) a polypeptide encoded by a polynucleotide that specifically hybridizes to a DNA comprising the nucleotide sequence set forth in SEQ ID NO:1 under highly stringent conditions of washing in 0.1 x SSC, wherein said polypeptide has activity of a

D-aminoacylase that acts on N-acetyl-D-amino acids to produce the corresponding D-amino acids, and fails to act on N-acetyl-L-tryptophan; N-acetyl-L-phenylalanine; N-acetyl-L-valine; N-acetyl-L-leucine; and N-acetyl-L-methionine; and

(d) a polypeptide having an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:2, wherein said polypeptide has activity of a D-aminoacylase that acts on N-acetyl-D-amino acids to produce the corresponding D-amino acids, and fails to act on N-acetyl-L-tryptophan; N-acetyl-L-phenylalanine; N-acetyl-L-valine; N-acetyl-L-leucine; and N-acetyl-L-methionine;

or a transformant expressively carrying a polynucleotide encoding said polypeptide or a vector comprising said polynucleotide or its culture medium, with N-acyl-DL-amino acid represented by the formula (I) or its salt:



wherein R<sub>1</sub> and R<sub>2</sub> may be identical or different and each represents a hydrogen atom or a substituted or unsubstituted hydrocarbon group; R<sub>2</sub> does not represent a hydrogen atom; and X is H, NH<sub>4</sub>, or a metal ion, and

(2) isolating and purifying the D-amino acid produced by the reaction between said polypeptide and said N-acyl-DL-amino acid.

12. (Currently Amended) The method of claim 11, wherein R<sub>1</sub> and R<sub>2</sub> in the formula (I) may be identical or different and each represents an alkyl, alkenyl, alkynyl, cycloalkyl, aryl, or aralkyl group, or the derivative thereof.

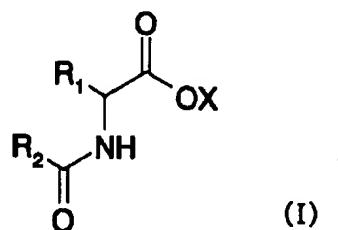
13. (Original) The method of claim 12, wherein R<sub>1</sub> is a  $\beta$ -methylindolyl, benzyl, thiomethylethyl, isopropyl, or 2-methyl-propyl group; and R<sub>2</sub> is a methyl, chloromethyl, phenyl, or aminomethyl group.

14. (New) A method for producing D-amino acids, the method comprising contacting a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2 with an N-acyl-DL-amino acid and isolating the D-amino acid produced by the reaction between said polypeptide and said N-acyl-DL-amino acid.

15. (New) A method for producing D-amino acids, the method comprising contacting a polypeptide consisting of the amino acid sequence set forth in SEQ ID NO:2 with an N-acyl-DL-amino acid and isolating the D-amino acid produced by the reaction between said polypeptide and said N-acyl-DL-amino acid.

16. (New) A method for producing D-amino acids, the method comprising:

(1) contacting a polypeptide having an amino acid sequence at least 95% identical to the amino acid sequence set forth in SEQ ID NO:2 with an N-acyl-DL-amino acid represented by the formula (I) or its salt:

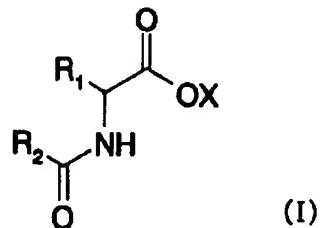


wherein R<sub>1</sub> and R<sub>2</sub> may be identical or different and each represents a hydrogen atom or a substituted or unsubstituted hydrocarbon group; R<sub>2</sub> does not represent a hydrogen atom; and X is H, NH<sub>4</sub>, or a metal ion, and

(2) isolating the D-amino acid produced by the reaction between said polypeptide and said N-acyl-DL-amino acid.

17. (New) A method for producing D-amino acids, the method comprising:

(1) contacting a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2 with up to 50 conservative amino acid substitutions with an N-acyl-DL-amino acid represented by the formula (I) or its salt:

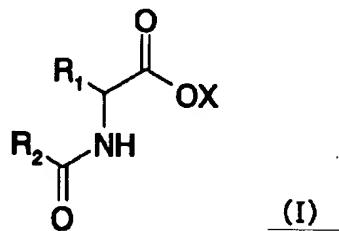


wherein R<sub>1</sub> and R<sub>2</sub> may be identical or different and each represents a hydrogen atom or a substituted or unsubstituted hydrocarbon group; R<sub>2</sub> does not represent a hydrogen atom; and X is H, NH<sub>4</sub>, or a metal ion, and

(2) isolating the D-amino acid produced by the reaction between said polypeptide and said N-acyl-DL-amino acid.

18. (New) A method for producing D-amino acids, the method comprising:

(1) contacting a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2 with an N-acyl-DL-amino acid represented by the formula (I) or its salt:

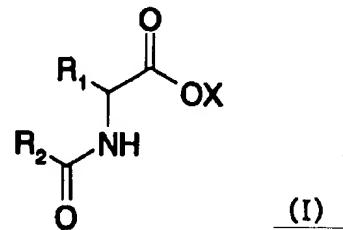


wherein R<sub>1</sub> and R<sub>2</sub> may be identical or different and each represents a hydrogen atom or a substituted or unsubstituted hydrocarbon group; R<sub>2</sub> does not represent a hydrogen atom; and X is H, NH<sub>4</sub>, or a metal ion, and

(2) isolating the D-amino acid produced by the reaction between said polypeptide and said N-acyl-DL-amino acid.

19. (New) A method for producing D-amino acids, the method comprising:

(1) contacting a polypeptide consisting of the amino acid sequence set forth in SEQ ID NO:2 with an N-acyl-DL-amino acid represented by the formula (I) or its salt:



wherein R<sub>1</sub> and R<sub>2</sub> may be identical or different and each represents a hydrogen atom or a substituted or unsubstituted hydrocarbon group; R<sub>2</sub> does not represent a hydrogen atom; and X is H, NH<sub>4</sub>, or a metal ion, and

(2) isolating the D-amino acid produced by the reaction between said polypeptide and said N-acyl-DL-amino acid.